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A systematic survey of antioxidant activity of 30 Chinese medicinal plants using the ferric reducing antioxidant power assay

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Abstract

The antioxidant activities and total phenolic contents of 30 Chinese medicinal plants were evaluated using the ferric reducing antioxidant power assay and the Folin–Ciocalteu method, respectively. The Chinese medicinal plants were extracted by the traditional method, boiling in water and also in 80% methanol. A significant and linear correlation coefficient between the antioxidant activity and the total phenolic content was found in both aqueous ($R^2 = 0.7917$) and methanol ($R^2 = 0.7584$) extracts. Phenolic compounds are thus a major contributor of antioxidant activity. Comparing the extraction efficiency of the two methods, the boiling water method extracted phenolic compounds more efficiently, and antioxidant activity of the extract was higher. It was found that the Chinese medicinal plants *Rhodiola sacra* Fu, the stem of *Polygonum multiflorum* Thunb. and the root of *P. multiflorum* Thunb. possessed the highest antioxidant activities and thus could be potential rich sources of natural antioxidants.

Keywords: Chinese medicinal plants; Antioxidant activity; Ferric-reducing antioxidant power assay; Phenolic content

1. Introduction

Reactive oxygen species (ROS), such as superoxide radical ($O_2^{\bullet -}$), hydroxyl radicals ($OH^{\bullet}$) and peroxyl radicals ($ROO^{\bullet}$), are produced as a part of normal metabolic processes. The oxidative damages caused by ROS on lipids, proteins and nucleic acids may trigger various chronic diseases, such as coronary heart diseases, atherosclerosis, cancer and aging (Madhavi, Deshpande, & Salunkhe, 1996). The health-promoting effect of antioxidants from plants is thought to arise from their protective effects by counteracting ROS. Indeed, epidemiological studies have demonstrated an inverse association between intake of fruits and vegetables and mortality from age-related diseases such as coronary heart diseases (Stephens et al., 1996) and cancer (La Vecchia, Altieri, & Tavani, 2001).

In view of these potential health benefits, there has been intensive research on natural antioxidants derived from plants. In particular, data from various studies (Cai, Luo, Sun, & Corke, 2004; Dragland, Senoo, Wake, Holte, & Blomhoff, 2003) indicate that Chinese medicinal plants contain a wide variety of natural antioxidants, such as phenolic acids, flavonoids and tannins, which possess more potent antioxidant activity than common dietary plants. Currently, much of the attention has been focussed on Chinese medicinal plants that are used for treating various diseases such as cancer (Cai et al., 2004) and inflammation (Schinella, Tournier, Prieto, de Buschiazzo, & Rios, 2002). However, apart from treating diseases, there is a group of Chinese medicinal plants used for safeguarding health. Such plants are under the 'pao' category, meaning supplementation (Ko, Mak, Chiu, & Poon, 2004), which is analogous to today's definition of functional foods. These medicinal

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plants have been consumed over thousands of years in China as a health tonic or as anti-aging remedies. The antioxidant property of some of the plants in this category, such as *Cordyceps sinensis* (Li, Li, Dong, & Tsim, 2001; Yamaguchi, Kagota, Nakamura, Shinozuka, & Kunitomo, 2000) and *Glycyrrhiza uralensis* Fisch. (Tang et al., 2004) have been assessed individually. However, most of the plants have not been assessed for their antioxidant activity. Traditionally, Chinese medicinal plants are boiled in water, and the extracts are used for consumption. Thus, the extracts derived from these plants may offer a safe and natural source of antioxidants that may be added to foods as additives or consumed directly as functional foods.

Phenolic compounds, such as flavonoids, phenolic acid, and tannins, are considered to be a major contributor to the antioxidant activity in Chinese medicinal plants. These antioxidants also possess diverse biological activities, such as anti-inflammatory, anti-carcinogenic and anti-atherosclerotic activities. These activities may be related to their antioxidant activity (Chung, Wong, Huang, & Lin, 1998). However, more scientific evidence is needed to verify the role of the phenolic compounds in the health-promoting and anti-aging effects of Chinese medicinal plants under the 'pao' category.

The aims of the present study were to measure the antioxidant activity of 30 selected Chinese medicinal plants under the 'pao' category, using the ferric-reducing antioxidant power assay (FRAP), to determine their total phenolic contents and to investigate the relationship between phenolic content and antioxidant activity. Furthermore, the efficiency of the traditional boiling water extraction method is evaluated by comparing its antioxidant activity and phenolic content to a more modern extraction method using 80% methanol.

2. Materials and methods

2.1. Chinese medicinal plants

The 30 Chinese medicinal plants were purchased from Beijing Tong-Ren-Tang drug retail outlet in Hong Kong. They are *Tremella fuciformis* Berk., *Alphinia oxyphylla* Mig., *Rhodiola sacra* Fu, *G. uralensis* Fisch., *Astragalus membranaceus* (Fisch.) Bge., *Polygonum multiflorum* Thunb., *Psoralea corylifolia* L., *Astragalus complanatus* R. Br., *Angelica sinensis* (Oliv.) Diels, *Morinda officinalis* How, *Atractylodes marcocephala* Koidz., *Dendrobium nobile* Lindl., *Paeonia lactiflora* Pall., *Epimedium brevicomum* Maxim., *Dipsacus japonicus* Miq., *Cynomorium songarium* Rupr., *Morus alba* L., *Dioscorea opposita* Thunb., *Eucommia ulmoides* Oliv., *Lilium brownii* F.E. Brown var. colchesteri Wils., *Curculigo orchioides* Gaerten., *Polygonatum odoratum* (Mill.) Druce, *Polygonatum sibiricum* Redoute, *Lycium barbarum* L., *Ophiopogon japonicus* Ker-Gawl., *Cistanche deserticola* Y.C. Ma, *Asparagus cochinchinensis* (Lour.) Merr, *Rehmannia glutinosa* Libosch. and *Cuscuta chinensis* Lam.

2.2. Chemicals

Iron (III) chloride 6-hydrate, iron (II) sulfate 7-hydrate and acetic acid were purchased from BDH (Dorset, England). Trolox and Folin–Ciocalteu's phenol reagent were purchased from Sigma–Aldrich (St. Louis, MO). Hydrochloric acid and methanol were purchased from Merck, Germany. 2,4,6-Tri(2-pyridyl)-*s*-triazine (TPTZ) was purchased from Fluka Chemie AG (Bushs, Switzerland).

2.3. Sample preparation

The samples were first ground to fine powder. For water extraction, 0.5 g of the fine powder was extracted with 10 ml of ultra-filtered water at 100 °C for 30 min in a water bath. For methanol extraction, 0.5 g of the powder was extracted with 10 ml of 80% methanol at 40 °C for 24 h. The samples were then cooled down to room temperature and centrifuged at 4500 rpm for 15 min. The supernatant was recovered and used for the FRAP assay and total phenolic analysis.

2.4. Ferric-reducing antioxidant power assay

The FRAP assay was carried out according to the procedure of Benzie and Strain (1996) with slight modification. Briefly, the FRAP reagent was prepared from acetate buffer (pH 3.6), 10 mmol TPTZ solution in 40 mmol HCl and 20 mmol iron (III) chloride solution in proportions of 10:1:1 (v/v), respectively. The FRAP reagent was prepared fresh daily and was warmed to 37 °C in a water bath prior to use. Fifty microliters of sample were added to 1.5 ml of the FRAP reagent. The absorbance of the reaction mixture were then recorded at 593 nm after 4 min. The standard curve was constructed using iron (II) sulfate solution (100–2000 μM), and the results were expressed as μmol Fe (II)/g dry weight of plant material. All the measurements were taken in triplicate and the mean values were calculated.

2.5. Determination of total phenolics

Total phenolics were estimated by the Folin–Ciocalteu method (Singleton & Rossi, 1965). Two hundred microliters of 1:10 diluted sample were added to 1 ml of 1:10 diluted Folin–Ciocalteu reagent. After 4 min, 800 μl of sodium carbonate (75 g/l) were added. After 2 h of incubation at room temperature, the absorbance at 765 nm were measured. Gallic acid (0–500 mg/l) was

used for calibration of a standard curve. The results were expressed as gallic acid equivalents (GAE)/g dry weight of plant material. Triplicate measurements were taken and mean values calculated.

3. Results and discussion

3.1. Total antioxidant power of Chinese medicinal plants measured by FRAP assay

The extracts of the 30 Chinese medicinal plants were tested for antioxidant activity using the FRAP assay, which is a simple assay that gives fast, reproducible results (Benzie & Strain, 1996). The FRAP is versatile and can be readily applied to both aqueous and alcohol extracts of different plants. In this assay, the antioxidant activity is determined on the basis of the ability to reduce ferric (III) iron to ferrous (II) iron. The results were expressed as μmol ferrous iron equivalents per g of sample.

Tables 1 and 2 show a wide range of differences in the antioxidant activity of the Chinese medicinal plants for the aqueous and methanol extracts, respectively. For the aqueous extracts, the antioxidant activity ranged from 1.9 to 532 μmol Fe (II)/g. *R. sacra* Fu was found to have the highest antioxidant activity (532 μmol Fe (II)/g), followed by the stem of *P. multiflorum* Thunb. (498 μmol Fe (II)/g) and the root of *P. multiflorum* Thunb. (343 μmol Fe (II)/g). For the methanol extracts, the antioxidant activity ranged from 2.5 to 480 μmol Fe (II)/g. *R. sacra* Fu was again found to have the highest antioxidant activity (480 μmol Fe (II)/g), followed by the stem of *P. multiflorum* Thunb. (302 μmol Fe (II)/g) and root of *P. multiflorum* Thunb. (149 μmol Fe (II)/g). According to the data obtained, the traditional Chinese medicinal plants were classified into four categories on the basis of their antioxidant activities: extremely high (>500 μmol Fe (II)/g), high (100–500 μmol Fe (II)/g), medium (10–100 μmol Fe (II)/g), and low (<10 mol Fe (II)/g). Under this classification, 1 plant showed extremely high antioxidant activity, 7 plants showed high antioxidant activity, 16 plants showed medium antioxidant activity and 6 plants showed low antioxidant activity in the aqueous extract. In the 80% methanol extract, it was found that no plants possessed extremely high antioxidant activity, but 3 plants had high antioxidant activity, 17 plants had medium antioxidant activity and 10 plants had low antioxidant activity.

Table 1
Antioxidant activities and phenolic contents of aqueous extracts of 30 Chinese medicinal plants

Scientific name	Total antioxidant activity	Total phenolic content
Alpinia oxyphylla Mig.	21.0 ± 0.4	3.97 ± 0.12
Angelica sinensis (Oliv.) Diels	35.2 ± 0.4	4.10 ± 0.03
Asparagus cochinchinensis (Lour.) Merr	9.2 ± 0.2	3.07 ± 0.06
Astragalus complanatus R. Br.	66.9 ± 1.4	10.3 ± 0.10
Astragalus membranaceus (Fisch.) Bge.	12.2 ± 0.4	5.27 ± 0.06
Atractylodes macrocephala Koidz.	17.4 ± 0.2	3.18 ± 0.04
Cistanche deserticola Y. C. Ma	77.7 ± 3.8	21.1 ± 0.12
Curculigo orchioides Gaerten.	52.0 ± 0.7	10.8 ± 0.06
Cuscuta chinensis Lam.	40.8 ± 1.5	9.10 ± 0.16
Cynomorium songaricum Rupr.	167 ± 3.7	19.0 ± 0.06
Dendrobium nobile Lindl.	17.6 ± 0.1	2.99 ± 0.09
Dioscorea opposita Thunb.	7.1 ± 0.1	2.37 ± 0.02
Dipsacus japonicus Miq.	170 ± 0.3	23.7 ± 0.29
Epimedium brevicomum Maxim.	166 ± 2.7	16.9 ± 0.30
Eucommia ulmoides Oliv.	39.3 ± 1.0	8.33 ± 0.10
Glycyrrhiza uralensis Fisch.	11.5 ± 0.2	14.0 ± 0.13
Lilium brownii F. E. Brown var. colchesteri Wils.	7.2 ± 0.1	2.58 ± 0.02
Lycium barbarum L.	21.2 ± 0.3	12.5 ± 0.10
Morinda officinalis How	18.9 ± 0.3	4.81 ± 0.09
Morus alba L.	52.7 ± 0.4	7.51 ± 0.23
Ophiopogon japonicus Ker-Gawl.	7.5 ± 0.2	2.50 ± 0.05
Paeonia lactiflora Pall.	117.4 ± 5.1	5.79 ± 0.05
Polygonatum odoratum (Mill.) Druce	1.9 ± 0.1	2.47 ± 0.06
Polygonatum sibiricum Redoute	50.0 ± 0.5	16.7 ± 0.16
Polygonum multiflorum Thunb. (root)	343 ± 1.7	33.9 ± 0.62
Polygonum multiflorum Thunb. (stem)	498 ± 2.0	42.3 ± 1.18
Psoralea corylifolia L.	27.7 ± 0.5	18.9 ± 0.22
Rehmannia glutinosa Libosch.	162 ± 1.3	32.1 ± 0.71
Rhodiola sacra Fu	532 ± 2.9	50.8 ± 1.38
Tremella fuciformis Berk.	4.9 ± 0.2	3.67 ± 0.10

Table 2
Antioxidant activities and phenolic contents of methanol extracts of 30 Chinese medicinal plants

Scientific name	Total antioxidant power	Total phenolic content
Alpinia oxyphylla Mig.	14.5 ± 0.4	3.94 ± 0.04
Angelica sinensis (Oliv.) Diels	27.3 ± 0.1	4.79 ± 0.05
Asparagus cochinchinensis (Lour.) Merr	9.3 ± 0.1	2.32 ± 0.01
Astragalus complanatus R. Br.	29.8 ± 0.2	6.77 ± 0.14
Astragalus membranaceus (Fisch.) Bge.	9.1 ± 0.6	3.87 ± 0.05
Atractylodes macrocephala Koidz.	9.0 ± 0.1	2.84 ± 0.05
Cistanche deserticola Y.C. Ma	62.8 ± 0.9	13 ± 0.12
Curculigo orchioides Gaerten.	12.3 ± 0.3	4.59 ± 0.03
Cuscuta chinensis Lam.	21.7 ± 0.7	6.69 ± 0.16
Cynomorium songaricum Rupr.	51.7 ± 1.9	12.3 ± 0.08
Dendrobium nobile Lindl.	9.3 ± 0.3	2.97 ± 0.06
Dioscorea opposita Thunb.	4.1 ± 0.1	1.44 ± 0.05
Dipsacus japonicus Miq.	95.6 ± 1.2	18.5 ± 0.17
Epimedium brevicomum Maxim.	81.8 ± 2.4	16.8 ± 0.10
Eucommia ulmoides Oliv.	20.4 ± 0.3	4.99 ± 0.05
Glycyrrhiza uralensis Fisch.	32.4 ± 0.9	14.5 ± 0.21
Lilium brownii F. E. Brown var. colchesteri Wils.	8.1 ± 0.5	1.45 ± 0.01
Lycium barbarum L.	21.7 ± 0.1	8.42 ± 0.09
Morinda officinalis How	6.1 ± 0.4	3.71 ± 0.07
Morus alba L.	34.8 ± 1.2	6.23 ± 0.02
Ophiopogon japonicus Ker-Gawl.	8.8 ± 0.1	1.31 ± 0.02
Paeonia lactiflora Pall.	90.6 ± 1.4	6.42 ± 0.03
Polygonatum odoratum (Mill.) Druce	3.6 ± 0.2	1.39 ± 0.05
Polygonatum sibiricum Redoute	27.5 ± 0.1	10.3 ± 0.07
Polygonum multiflorum Thunb. (root)	302 ± 3.0	24.2 ± 0.22
Polygonum multiflorum Thunb. (stem)	149 ± 6.7	17.7 ± 0.20
Psoralea corylifolia L.	72.7 ± 1.0	20.5 ± 0.13
Rehmannia glutinosa Libosch.	67.2 ± 1.7	15.8 ± 0.06
Rhodiola sacra Fu	480 ± 2.9	36.2 ± 0.98
Tremella fuciformis Berk.	2.5 ± 0.2	2.79 ± 0.01

In this study, the plants were extracted using a traditional method in Chinese medicine, by boiling in water. The other sample set was extracted with a more modern method, using 80% methanol. The average antioxidant activity of the boiling water extracts (91.8 μmol Fe (II)/g) was found to be higher than that of the methanol extracts (58.8 μmol Fe (II)/g). Also it was found that 23 of the 30 medicinal plants demonstrated a higher antioxidant activity in their boiling water extracts than in the methanol extracts. Therefore, the efficiency of the boiling water in extracting antioxidant activity is higher than that of the methanol extracts.

From the data obtained, three medicinal plants which are potentially rich sources of natural antioxidants were identified: *R. sacra* Fu, the stem of *P. multiflorum* Thunb. and the root of *P. multiflorum* Thunb. These three Chinese medicinal plants demonstrated the highest antioxidant activity and total phenolic content among the 30 plants tested. *R. sacra* Fu was reported to show anti-radiation effects and could improve learning and memory (Ming, Xia, & Zheng, 1988). From this plant, Ohsugi et al. (1999) identified eight phenolics that show strong superoxide scavenging activity. The root of *P. multiflorum* Thunb., known as He Sou Wu, is a famous traditional Chinese medicinal plant that has been used as a tonic and an anti-aging agent. Gallic acid, catechin and stilbene glycosides isolated from this plant have shown strong DPPH radical-scavenging activities (Chen, Wang, Rosen, & Ho, 1999). Interestingly, it is found that the stem of *P. multiflorum* Thunb., known as Ye Jiao Teng, showed an even more potent antioxidant capacity than its root. In addition, no prior report on the antioxidant activity of the stem of *P. multiflorum* Thunb. was found in the literature. Thus, it is possible that the stem of *P. multiflorum* Thunb. might exhibit a greater antioxidant-mediated anti-aging effect than the root of *P. multiflorum* Thunb., its more famous counterpart. Furthermore, the stem of *P. multiflorum* Thunb. is available at a low cost and thus provides an economic source of natural antioxidants for use as supplements or food additives. Compounds responsible for the potent antioxidant activity in the stem of *P. multiflorum* Thunb. are currently under investigation.

Over 8000 species of medicinal plants have been described in traditional Chinese medicine. In order to facilitate the search for Chinese medicinal plants with high antioxidant activity, the Chinese medicinal plants in this study were further classified and their antioxidant activities compared according to the traditional classification in Chinese medicine. In Chinese medicine theory, it is believed that the maintenance of whole body balance, known as yin-yang, is able to prevent the occurrence

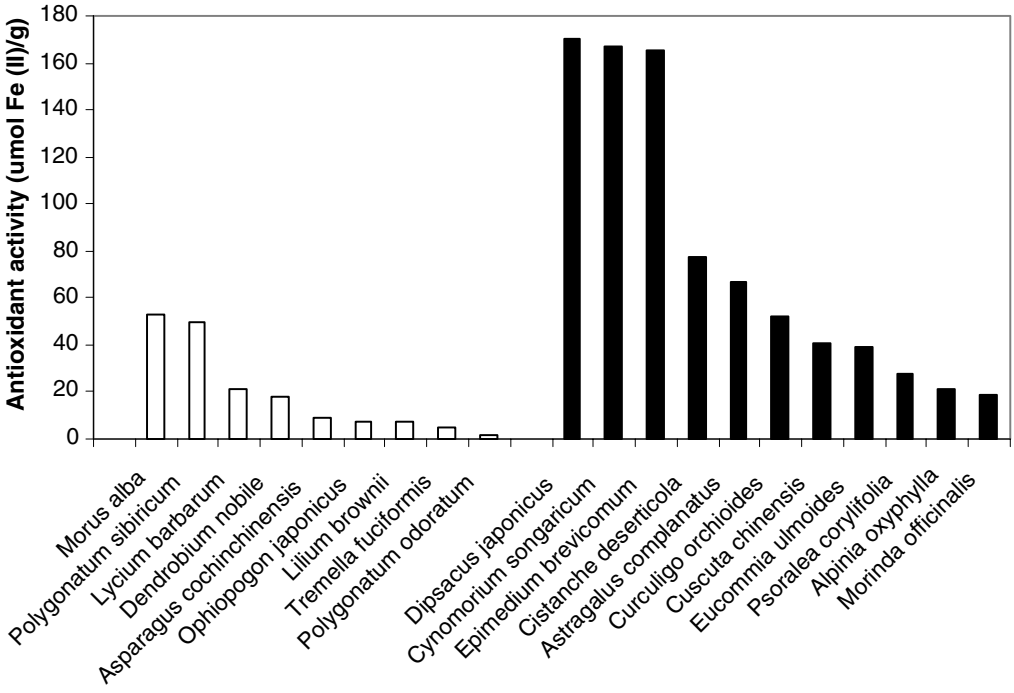

Fig. 1. Antioxidant activity of Yin-nourishing and Yang-invigorating Chinese medicinal plants (boiling water extracts). □, Yin-medicinal plants; ■, Yang-medicinal plants.

of various diseases and even slows the aging process. In this study, 9 Chinese medicinally plants are grouped under 'Yin-nourishing' and another 11 plants grouped under 'Yang-invigorating'. Fig. 1 shows that Yang-invigorating Chinese medicinal plants, in general, possess more potent antioxidant activity than Yin-nourishing plants, although there is a narrow range of overlapping values. Therefore, yang-invigorating Chinese medicinal plants may be a more useful source of natural antioxidants. This finding is in agreement with the results of Ko et al. (2004) who found that Yang plants possessed a higher antioxidant activity than Yin plants.

3.2. Total phenolic content of Chinese medicinal plants

The phenolic contents of the aqueous and methanol extracts of Chinese medicinal plants were tested using the diluted Folin–Ciocalteu reagent. The variation of total phenolic content was also significant (Tables 1 and 2). The phenolic contents of aqueous extracts varied from 2.4 to 50.8 mg GAE/g. *R. sacra* Fu (50.8 mg GAE/g), the stem of *P. multiflorum* Thunb. (42.3 mg GAE/g) and the root of*P. multiflorum* Thunb. (33.9 mg GAE/g) were found to have the highest phenolic contents. Other plants with significant phenolic contents (>20 mg GAE/g) were *C. deserticola* Y.C. Ma, *D. japonicus* Miq. and *R. glutinosa* Libosch. The GAE of methanol extracts ranged from 1.3 to 36.4 mg GAE/g. A similar pattern was observed in methanol extracts. *R. sacra* Fu had the highest phenolic content (36.4 mg GAE/g), followed by the stem of *P. multiflorum* Thunb. (24.2 mg GAE/g) and *P. corylifolia* L. (20.5 mg GAE/g). The roots of *P. multiflorum* Thunb., *R. glutinosa* Libosch., *D. japonicus* Miq., and *E. brevicomum* Maxim. were also found to have relatively high phenolic contents (>15 mg GAE/g).

On comparing the efficiency of extraction with boiling water and 80% methanol, a trend similar to the antioxidant activity was found. The method using boiling water showed a greater efficiency in the extraction of phenolic compounds than that with 80% methanol. The mean of the total phenolic content of water extracts (13.3 mg GAE/g) was also found to be higher than that of the methanol extracts (9.3 mg GAE/g). 26 out of 30 medicinal plants showed a higher phenolic content in boiling water extracts than in methanol extracts, with the exception of *P. corylifolia* L., *P. lactiflora* Pall., *A. sinensis* (Oliv.) Diels and *G. uralensis* Fisch. in which the phenolic content of the aqueous extracts was lower than that of the methanol extracts. Thus, it was concluded that boiling water is a more efficient way of extracting phenolic compounds from medicinal plants than 80% methanol. Although there are many components that may contribute to antioxidant activity, the predominant contributors to antioxidant activity, such as phenolic compounds, are better extracted using boiling water than 80% methanol. The greater efficiency of boiling water in extracting the phenolic compounds would be expected to result in higher antioxidant activity. Due to the merits of much lower toxicity of water than that of methanol and yet superior extraction

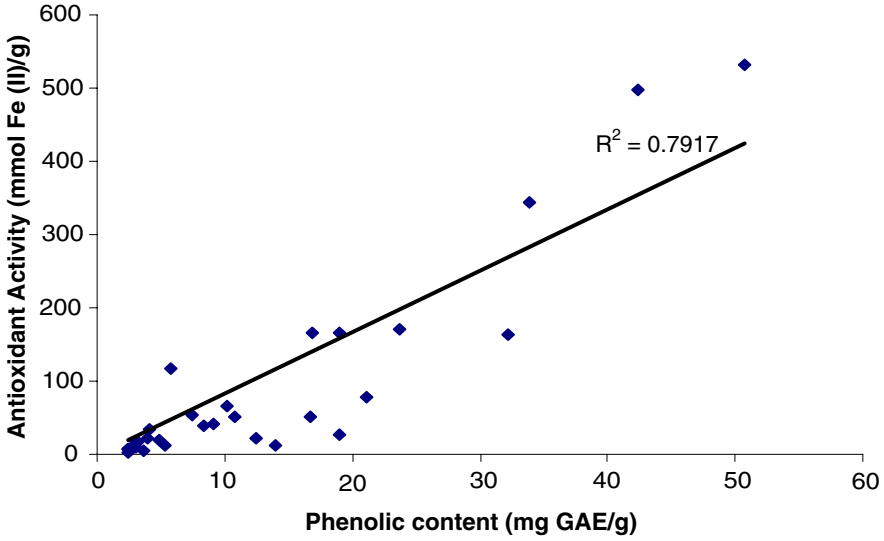

Fig. 2. Correlation between the antioxidant activity and phenolic content for aqueous extracts. GAE: gallic acid equivalents.

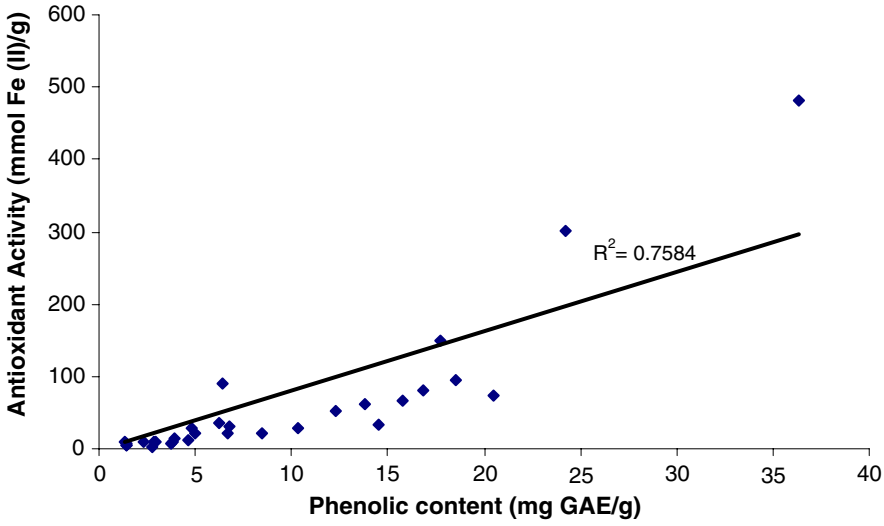

Fig. 3. Correlation between the antioxidant activity and phenolic content for methanol extracts. GAE: gallic acid equivalents.

efficiency, it offers a better choice in obtaining antioxidant-rich extracts.

3.3. The correlation between antioxidant activity and total phenolic content

The correlation coefficient (R^2) between the antioxidant activity and the total phenolic content of the 30 Chinese medicinal plants was determined (Figs. 2 and 3). The antioxidant activity and the total phenolic content showed a good correlation in both the aqueous ($R^2 = 0.7917$) and methanol ($R^2 = 0.7584$) extracts. Therefore, the presence of phenolic compounds contributed significantly to the antioxidant activity of the Chinese medicinal plants under the 'pao' category. High phenolic content is thus an important factor in determining the antioxidant activity of Chinese medicinal plants. This result is in agreement with previous reports that the phenolic compounds contribute significantly to the antioxidant activity in different Chinese medicinal plants (Cai et al., 2004; Tang et al., 2004).

In conclusion, the antioxidant activity and total phenolic content of the 30 Chinese medicinal plants in the 'pao' category were evaluated. The Chinese medicinal plants, in general, showed high antioxidant activities and phenolic content. In particular, *R. sacra* Fu, the stem of *P. multiflorum* Thunb. and the root of *P. multiflorum* Thunb. were found to be most potent. In addition, a significant and linear relationship existed between the antioxidant activity and phenolic content, indicating that phenolic compounds are major contributors to antioxidant activity. Given the possible antioxidative, anticarcinogenic and anti-atherosclerotic effects of the phenolic compounds, the Chinese medicinal plants in the

'pao' category are likely to play a major role in health-promoting and anti-aging activities. Further in vivo studies on the phenolic compounds would be required to establish the functionality of these herbs.

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